

CHITINOUS FIBRILS IN THE LORICA OF THE
FLAGELLATE CHRYSOPHYTE *POTERIOCHROMONAS*
STIPITATA (SYN. *OCHROMONAS MALHAMENSIS*)

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ABSTRACT

Ordered microfibrils are formed on the membrane of the cytoplasmic tail of the alga *Poteriochromonas* after attachment to a substrate. The ultrastructure of native and extracted stalk fibrils was studied with electron microscope methods. In addition, the structural polysaccharide was characterized by hydrolyses, separation of the monomers by thin-layer chromatography, gas-liquid chromatography and amino acid analysis, and by X-ray diffraction. The alkali-resistant fibrils yielded mostly glucosamine upon extensive hydrolysis, and showed X-ray diffraction patterns similar to those of fugal chitin. It is concluded that the resistant core of the fibrils is chitinous.

In the literature, there is a strong tendency to interpret any microfibrillar structures in cell walls of higher plants, but also of lower organisms, as cellulosic, more so when they are deposited in ordered arrangements. Yet, in principal, any linear polysaccharide of high degree of polymerization may appear fibrillar in the electron microscope (16, 31, 51, 52).

Identification of the structural polysaccharide(s) of a microfibril by biochemical methods is necessary to establish its chemical nature. This study provides a clear example of this identification for the lorica microfibrils of the unicellular flagellate *Poteriochromonas stipitata*, which have repeatedly been described to be cellulosic (32, 33, 61).

Our finding that these fibrils are chitinous rather than cellulosic also revives the old question of chitin in algal cell walls hitherto only confirmed for diatoms (5, 16, 46) and not even mentioned in most textbooks on algae (e.g., references 11 and 12).

MATERIALS AND METHODS

*Algal Cultures and Preparation
of Lorica Material*

Poteriochromonas stipitata (Scherffel), syn. *Ochromonas malhamensis* (Pringsheim), strain no. 933-1a from the Algal Culture Collection of the Institute of Plant Physiology, University of Göttingen, Göttingen, Germany, was grown axenically in sterile medium (33). The algae were cultivated at 25°C in a light-thermostat (F. Kniese, Marburg-Marbach, Germany) under continuous illumination of 15,000 lx, according to the conditions described (39). The algal suspensions, 200 ml per culture tube, were aerated by bubbling air through the tubes. A higher yield of loricae was induced by stopping the air stream for 3 h twice daily from the 3rd to the 7th day of culture. This results in the formation of many small colonies of algae attached to one another at the basal parts of their loricae. The colonies were routinely monitored by light microscopy, and any cultures found to be contaminated by fungi or bacteria were excluded from analysis. The cells and loricae were collected by centrifu-

gation (20 min at 4,000 g), and the pellets were stored at -30°C until used for further extractions.

Extractions

After 10–12 days of culture, the cells undergo autolysis (1). The loricae were not structurally disintegrated during this process (W. Herth, unpublished observations) and were collected free from cellular material after the autolysis. This material was used in parallel with the material from the 7-day-old cultures. Extractions with methanol, methanol/chloroform, and various concentrations of HCl, trifluoroacetic acid (TFA), and/or NaOH were performed as described (22, 24).

Hydrolyses, Chromatography, and X-Ray Diffraction

Technical details of the hydrolyses with 6 N HCl or 2 N TFA, chromatographic procedures (thin-layer chromatography, gas liquid chromatography, and amino acid analyses), and X-ray diffraction were carried out as described earlier (21–24).

Staining of Loricae

Freshly synthesized loricae on Formvar-coated grids for electron microscopy (see below) were incubated for 10 min in a solution of cationized ferritin which was added to the medium in the concentration suggested by the manufacturer (Yeda Research and Development Co., Ltd., Rehóvot, Israel), and excess ferritin was removed by washing with distilled water. The dried grids were examined in the electron microscope without further staining. The same procedure was used with 0.1% ruthenium red. Calcofluor white ST staining (American Cyanamid Co., Wayne, N. J.) was performed with a 0.1% solution in water (10 min) applied to isolated loricae and was examined in a Reichert Zetopan fluorescence microscope. Chlor-zinc-iodine staining was carried out as usual (16). For birefringence determination, we used a Reichert Zetopan microscope.

Electron Microscopy

For structural observations on fresh loricae, algae from the 5th day of culture (stationary growth phase, cf. reference 55) were allowed to attach to Formvar-coated copper grids (100 mesh) for electron microscopy. After 2 h, during which loricae were synthesized, the algal protoplasts were washed out of the loricae with distilled water, and the loricae attached to the grid were negatively stained with 2% phosphotungstic acid, pH 7.2. For metal shadowing, gold/palladium at an angle of 40° (Siemens Kleinbedampfungsanlage) was used. Electron micrographs were taken with a Siemens Elmiskop 101.

RESULTS

Ultrastructure

The native lorica of *Poteriochromonas* consists

of a branched foot part, a stalk up to $50\ \mu\text{m}$ long, and a cuplike upper part. A detailed description of its structure will appear elsewhere (cf. also references 32, 33, 61, and 62).¹ In the stalk region, the fibrils are helically ordered (Fig. 1), the helical pitch is rather steep, and the upper part opens into the cuplike region where the fibrils are arranged more randomly. The rather broad fibrillar ribbons in the stalk consist of bundles of thinner fibrils which are laterally associated. Fibrillar widths in the stalk are $15\text{--}25\ \text{\AA}$; the length cannot be measured since only occasionally is a fibrillar end detectable. We have been unable to trace an individual fibril over its entire length in such ribbon-like associations.

Analyses

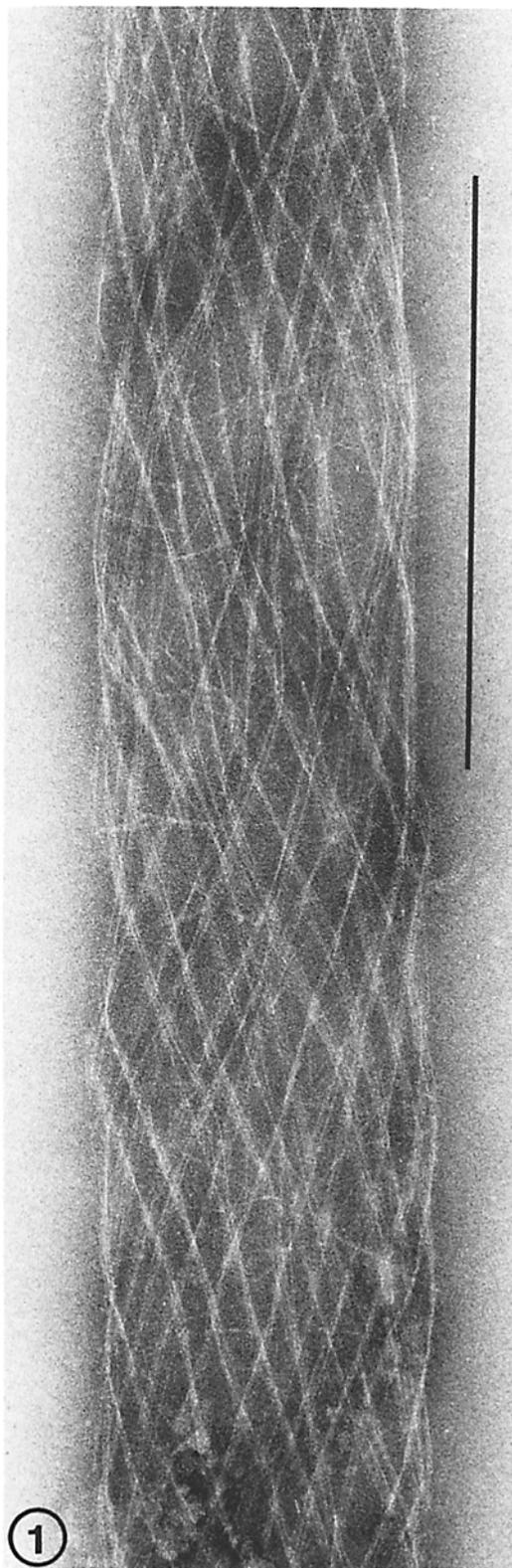
The density of the isolated, lipid-extracted loricae is near 1.5, i.e. they do not sediment in chloroform (20 min at 4,000 g) but remain evenly distributed in the solvent.

Native and purified loricae stain with the fluorescent dye Calcofluor white. Native loricae bind cationized ferritin; the fibrils appear negatively stained with this method. The loricae also stain with ruthenium red. After acidic and alkaline extractions, samples stain a violet color with chlor-zinc-iodine. The chlor-zinc-iodine dichroism is positive.

The *Poteriochromonas* fibrils resist dilute acidic extraction. After liquid extraction and extraction with 1 N HCl (30 min at 100°C), the typical arrangement of the fibrils in the stalk and cup regions of the lorica is undistorted, whereas the foot part of the lorica is no longer detectable. The fibrils also resist higher concentrations of HCl. They are not destroyed by 10-min treatment with 12 N HCl at 20°C , and are partially degraded after such treatment for 2 h (Fig. 3). In 10 N sulfuric acid, the fibrils are dissolved within 10 min., leaving no detectable residue. Short rodlike elements similar to the degradation products in 12 N HCl (Fig. 3) reprecipitate when the sulfuric acid with the dissolved material is diluted 10 times again.

The fibrils of the stalk- and cup-regions of the lorica are also very resistant to alkali extractions. After 2 h at 100°C in 1.5 N NaOH, the resistant material consists of stalk- and cup-fragments and short rodlike elements. The regular structural arrangement of the fibrils in the stalk region is still

¹ Herth, W., G. Röderer, and E. Schnepf. Manuscript in preparation.



recognized in the stalk fragments in metal-shadowed preparations (Fig. 2). The broad, ribbon-like and laterally associated fibrillar bundles are arranged helically. The more randomly oriented individual internal fibrils which stabilize the arrangement are seen clearly. The fibrils must be attached to each other by alkali-resistant linkages. During treatment with 5 NaOH (2 h at 100°C), the fibrils are further broken down into short rods (Fig. 4).

The X-ray diffraction pattern of unextracted lorica material is very diffuse. After short acidic extraction (1 N HCl, 30 min at 100°C), the reflections are still few and diffuse. No cellulosic reflections are detected. The main monosaccharide detected in native lorica material after hydrolysis with 6 N HCl (24 h at 110°C) is glucose. However, if hydrolyses are performed with 2 N TFA, a procedure which is less destructive for acid-sensitive sugars, the presence of more sugars in hydrolysates of native *Poteriochromonas loricae* is revealed: glucose is still the main monomer, but galactose, mannose, xylose, and a fast moving sugar, probably rhamnose, are also present (Fig. 6). In addition, a constituent reactive with both ninhydrin and aniline phthalate is detected; it has the same relative mobility as glucosamine upon thin-layer chromatography.

In total hydrolysates of fibrillar material (6 N HCl, 48 h at 110°C) resistant to extractions with 1 N HCl (30 min at 100°C) and 1.5 N NaOH (30 min at 100°C), the sugar monomers were determined by gas-liquid chromatography of the alditol acetate derivatives (Fig. 7). With this method, the amount of glucose is calculated to be 53% of the total sugars in the hydrolysate; glucosamine is nearly 40% of the total sugars. Only small amounts of xylose (2%), mannose (3%), and galactose (2%) are detected.

After 6-h extraction with 2 N TFA at 110°C, the lorica fibrils are only partially broken down (Fig. 5). The resistant material consists of various lengths of very thin fibrils which show lateral associations. Total hydrolysates of this TFA-resistant material (6 N HCl, 48 h at 110°C) contain almost exclusively glucosamine as determined by thin-layer chromatography and by amino acid analysis. Only trace amounts of glucose, no other sugars

FIGURE 1 Stalk region of the lorica of *Poteriochromonas stipitata*. Fibrillar bundles are helically arranged and cross-linked by individual thin fibrils. Negative stain. Bar, 1 μm . $\times 80,000$.

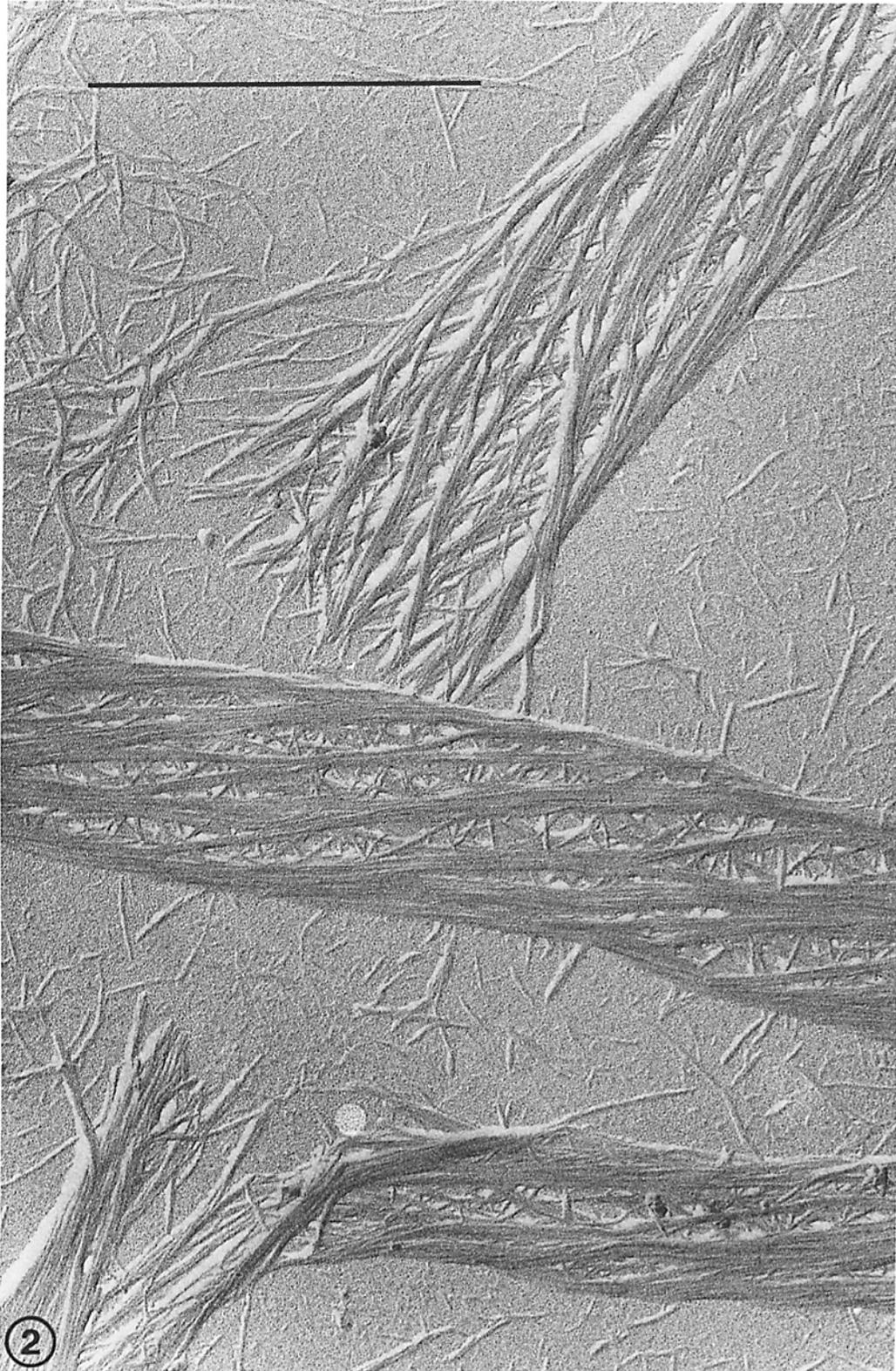


FIGURE 2 Fractions shadowed with gold/palladium after extractions with boiling 1 N HCl (1 h) and 1.5 N NaOH (2 h). The loricae are partially degraded into short rods, but most of the material still has retained the fibrillar arrangement typical for the stalk. The fibrillar bundles are located external to the fibrils that cross-link the bundles. Bar, 1 μm . $\times 60,000$.

and no amino acids are detected in these hydrolysates.

The X-ray diffraction pattern obtained from the TFA-extracted fibrillar material (Fig. 8a) is in agreement with our other finding that the most resistant part of the *Poteriochromonas* fibrils is a polymer of glucosamine. This pattern is very similar to the X-ray diffraction pattern obtained from purified fungal chitin (Fig. 9b) but different from that obtained from cellulose (Fig. 8b). Similar, but even sharper, chitin-like X-ray diffraction patterns are obtained from the *Poteriochromonas* fibrillar material when combinations of crude acidic and alkaline extractions are analyzed (Fig. 9a). The *Poteriochromonas* pattern differs from the chitin pattern in one additional reflection (arrow in Fig. 9a), probably still indicating the presence of matrix materials.

DISCUSSION

The occurrence of chitin fibrils in algae, although postulated repeatedly (for review, see references 16, 42, 52, and 56), has so far been definitely demonstrated only for diatoms (5, 46) where a very pure and crystalline chitin has been reported. With *Poteriochromonas*, no extraction method was found that leaves a crystalline residue such as that in the diatoms. The question of whether the fibrils consist of a common α -chitin comparable to crustacean and fungal chitin or whether the chitin is similar to diatom chitin remains unanswered (for discussion, cf. references 5, 9, and 57). The low crystallite sizes to be expected from thin fibrils such as those of *Poteriochromonas* may explain the diffuse X-ray patterns obtained from native and extracted materials. The fibrils also may contain nonacetyl-glucosamine sugars which could distort the lattice (10, 52) and cause additional reflections (Fig. 9). There is no hint of the existence of any other crystalline polysaccharide in the fibrils. X-ray diffraction patterns corresponding to a cellulose I or cellulose II pattern were never observed. This is probably not due to the extraction procedures used, since with other materials cellulose has been demonstrated after similar extractions even when obscured by other components in the native material (cf. references 22–24). There are also no reflections indicative of a crystalline β -1,3-glucan (cf. references 22, 28, 34, and 36).

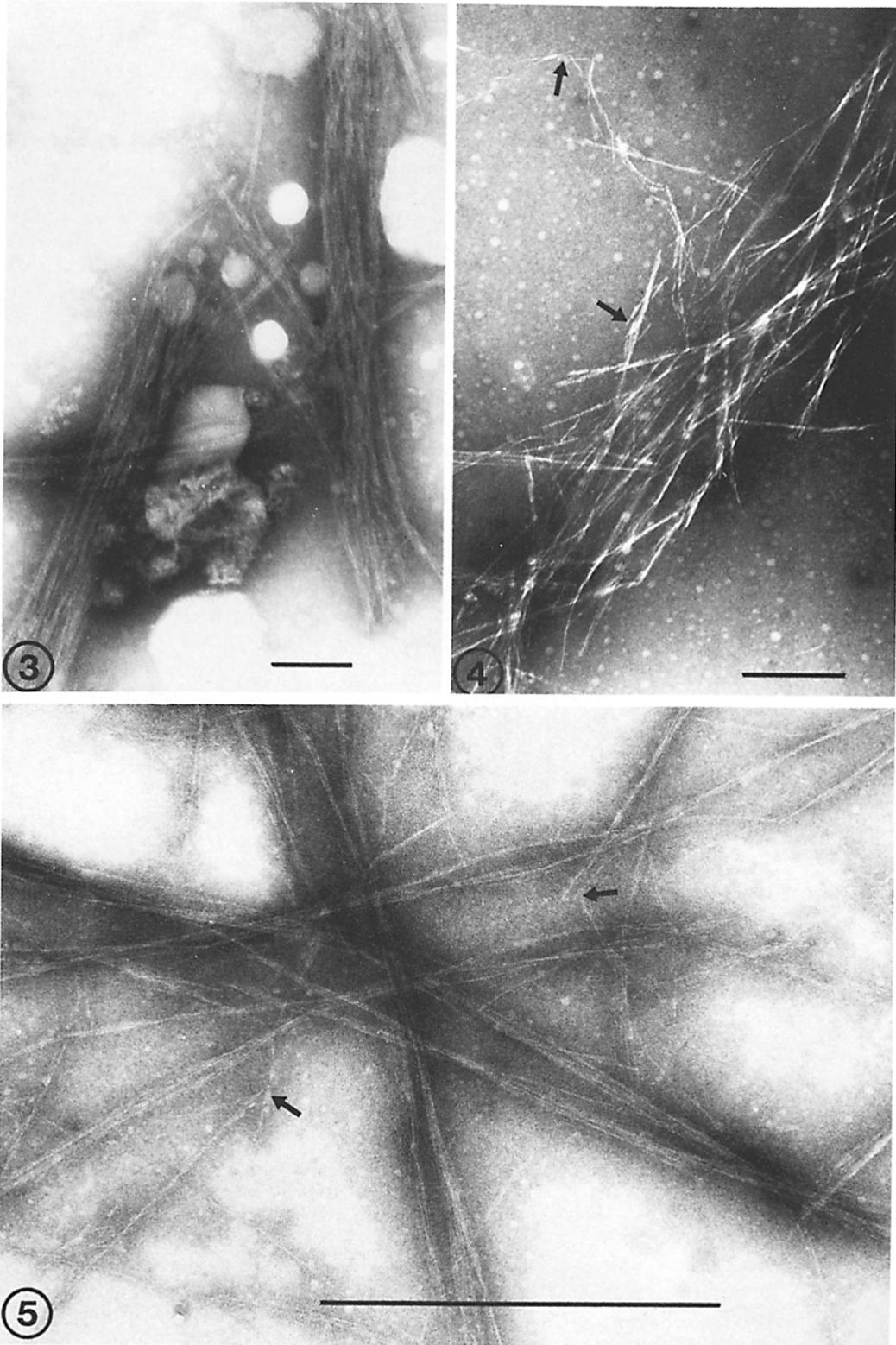
Glucose, other sugars, and the amino acids detected in less extracted samples were almost totally removed by acid hydrolysis in 2 N TFA. Such

treatment has been found not to degrade cellulose (21, 24, 49), but rather to hydrolyze matrix materials. Thus the nonglucosamine monomers appear to be derived from the less resistant matrix materials of the lorica. These are probably identical with the acidic polysaccharides having the same sugar composition and a high content of glucuronic acid that have been described to be secreted into the culture medium (29). Ruthenium red stainability and binding of cationized ferritin support the assumption of an acidic matrix surrounding the fibrils. Mixed linkages are most probable, since, in fungi, the glucose-rich matrix surrounding the chitin fibrils often contains various linkage types (for review, see references 3, 4, and 43).

Calcofluor stainability of the fibrils is often interpreted as indicating either cellulose or chitin (e.g., reference 44), but is by no means specific. In control experiments, we found that even agar is stained. The chlor-zinc-iodine stain seems also not to be a reliable test since it reacts with various structural polysaccharides (e.g., references 16 and 22).

The insolubility of the *Poteriochromonas* lorica fibrils in 12 N HCl (see Fig. 3) and 5 N NaOH (see Fig. 4) suggests that they are highly acetylated (16, 35, 52). Deacetylated chitin and chitosan is markedly less resistant (34). Direct measurements of the degree of acetylation were not yet possible due to the small amounts of material. Under acidic hydrolytic conditions, the liberated *N*-acetyl-glucosamine is rapidly degraded to glucosamine so that in chitin hydrolysates mostly glucosamine is obtained. The glucosamine values obtained are probably underestimates, as the recovery of glucosamine relative to other sugars by gas-liquid chromatographic analysis is rather low (Stadler, J. Personal communication).

The widths of the *Poteriochromonas* fibrils, 15–25 Å, are in the range of the widths of fungal chitin fibrils (53) and arthropod cuticle chitin crystallites (48). The electron microscope appearance of the fibrils in the lorica of *Poteriochromonas*, however, is also very similar to that of various cellulosic materials (e.g., references 6–8, 13, 17, 19, 20, 24, 47, and 50), though different from that of the extremely crystalline cellulose ribbons of, e.g., *Valonia* and *Glaucocystis* (18, 52, 54, 60). In the region of the *Poteriochromonas* stalk, the individual fibrils form ribbon-like broader units by lateral association (“primary fibrils of the stalk” according to reference 62), in which the individual thin fibrils are still recognizable with



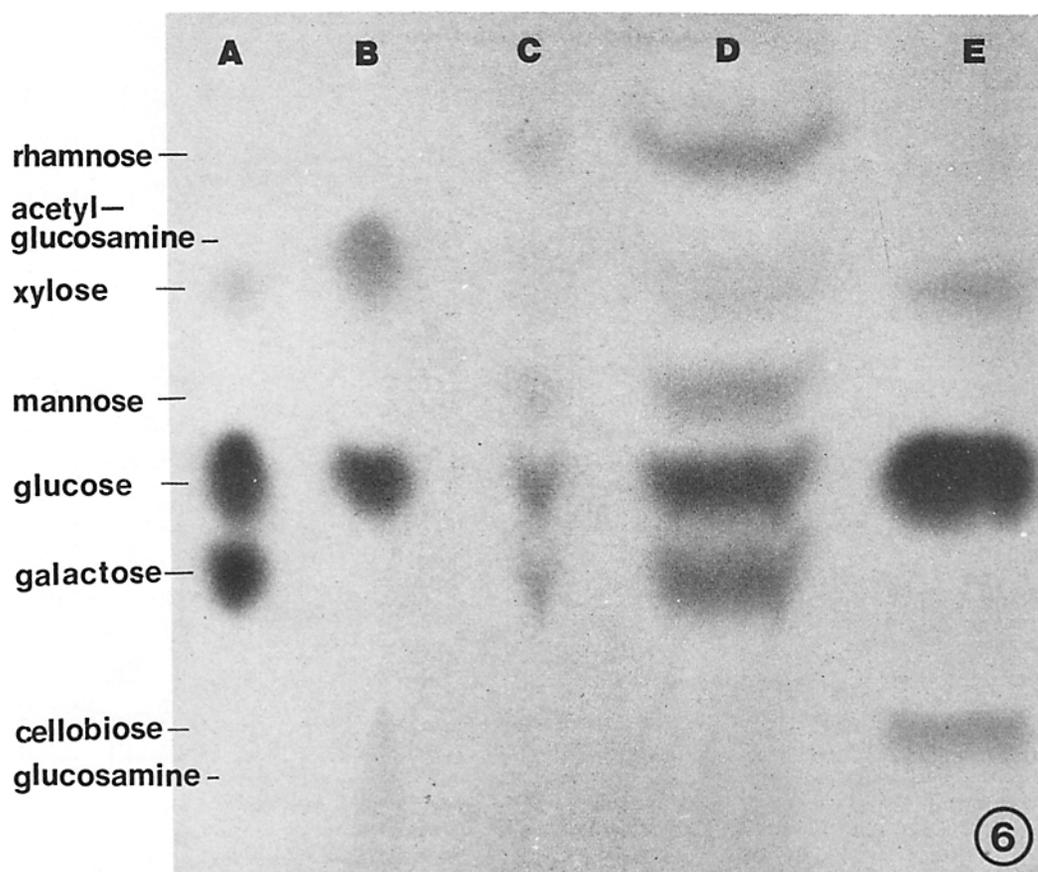


FIGURE 6 Thin-layer chromatographic separation of the sugar monomers released by 2 N TFA partial hydrolysis (6 h at 110°C) from an unextracted lorica fraction. Lane A sugar standards: galactose, glucose, and xylose. Lane B sugar standards: glucosamine, glucose, and *N*-acetyl-glucosamine. Lanes C and D hydrolysate applied as spot and as line. Lane E sugar standards: cellobiose, glucose, and xylose. Aniline phthalate spray reagent for sugar detection.

negative staining. This is found for native material without degradative treatments, e.g., enzymatic digestions (cf. reference 14 for *Valonia* ribbons) or ultrasonic treatments (18, 20, 47). This strong

tendency for lateral associations is not so marked but is also observed with the elementary or sub-elementary fibrils of cellulose (2, 6-8, 13, 17, 20, 24-26, 47).

FIGURE 3 Treatment with 12 N HCl at 20°C for 2 h leaves only short rods with a tendency to associate laterally. Negative stain. Bar, 0.1 μm . \times 125,000.

FIGURE 4 Lorica material resistant to extraction with boiling 5 N NaOH (2 h). Partial breakdown of the fibrils is seen (arrows). Short rods are the main breakdown product. Negative stain. Bar, 0.2 μm . \times 80,000.

FIGURE 5 Acid-resistant material of the lorica of *Potriochromonas* after 6 h in 2 N TFA at 110°C. The fibrils are partially broken down (arrows). They no longer show the arrangement typical for native loricae. Individual long fibrils are associated laterally into bundles. Negative stain. Bar, 0.5 μm . \times 125,000.

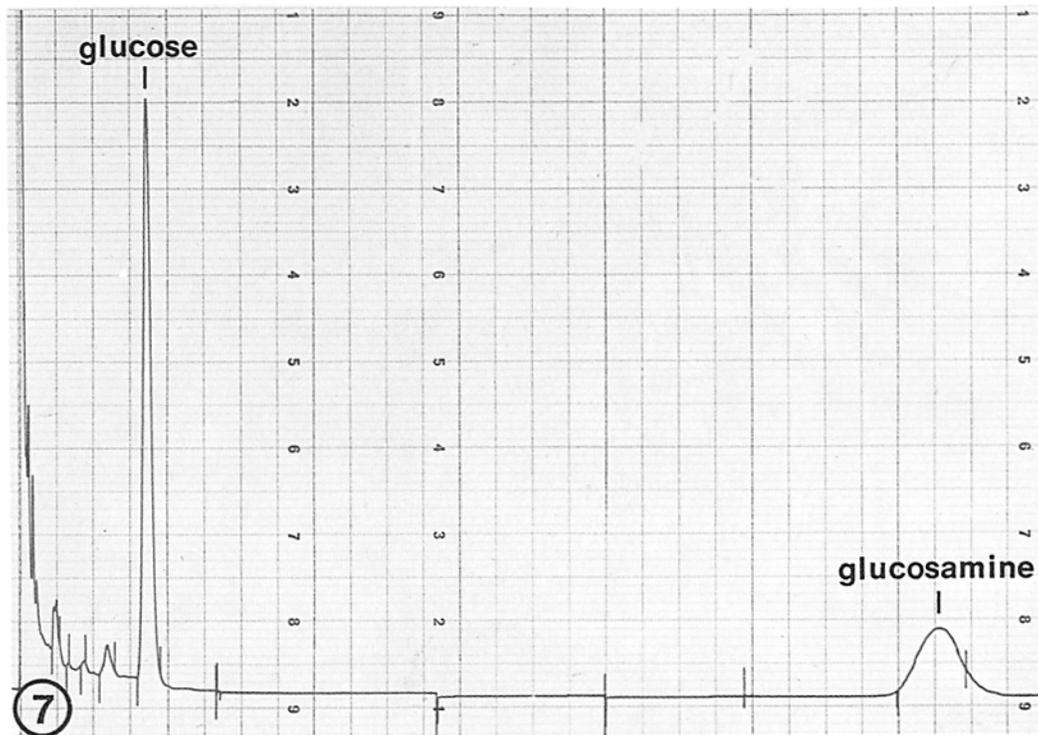


FIGURE 7 Gas-liquid chromatographic separation (200°C, isothermic) of the alditol acetate derivatives of the sugars in a total hydrolysate (6 N HCl, 48 h at 110°C) of a lorica fraction (extracted sequentially with 1 N HCl, 1 h at 100°C, and 5 N NaOH, 1 h at 100°C). The main peaks correspond to glucose- and glucosamine-derivatives.

The noncellulosic structural polysaccharides found in siphonous green algae (51, 52), some acidic algal polysaccharides (e.g., reference 52), and β -1,3-glucans (21, 22, 28, 31, 45) are also fibrillar with dimensions similar to those of the *Poterochromonas* fibrils. Fibrillar appearance, even in combination with acid- and alkali-resistance, can not be regarded as proof of a cellulosic nature of a component in question. With this in mind, other fibrils said to be cellulosic, e.g. of the closely related organism *Dinobryon* (11, 15, 27, 30) and other *Chrysophyceae* (e.g., reference 37 and 38), should be reinvestigated.

We conclude that in *Poterochromonas* the lorica contains a structural polysaccharide similar to chitin. This polysaccharide is associated with other, less resistant and chemically different polysaccharides. The effectiveness of the TFA-extraction and the obtained X-ray diffraction patterns after crude extractions rather rule out a glucose-

glucosamine heteropolymer for the most resistant part of the fibrils (for similar effects of extractions on cellulose, cf. reference 10). Covalent linkages of the chitinous polymer with the other components, as reported, for example, for the components of primary cell walls (40, 41), however, can not be excluded as the extraction conditions are certainly hydrolytic.

The reported unidirectional tip growth of the *Poterochromonas* fibrils (32, 33, 62) is in agreement with the evidence for chitin synthesis in vitro with fungal chitin synthetase (58, 59).

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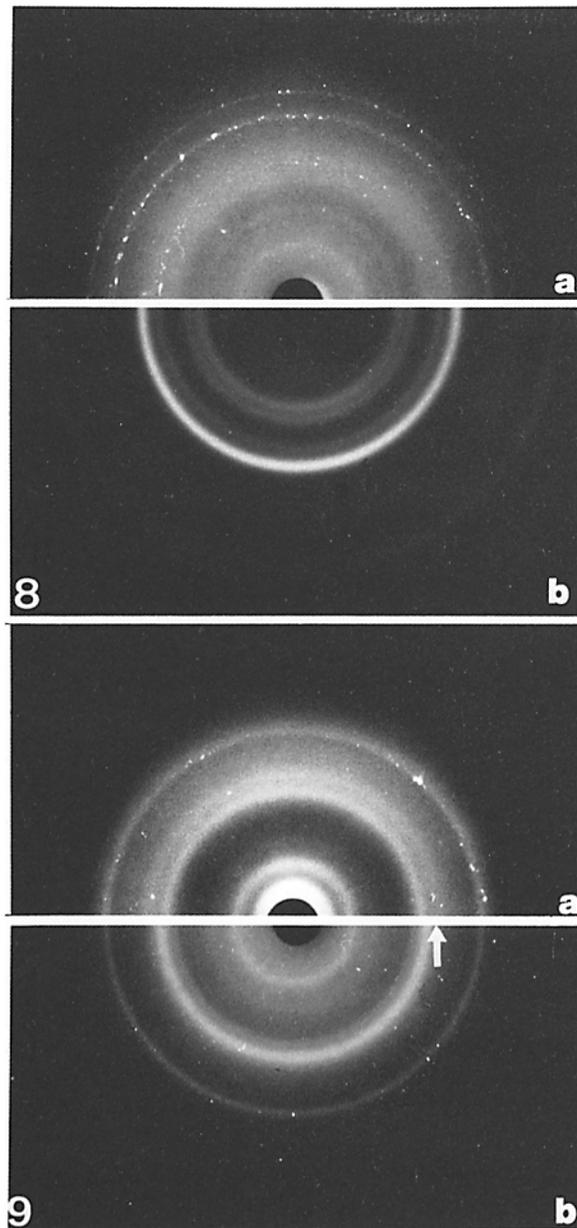


FIGURE 8 a and b X-ray diffraction pattern of the TFA-extracted polysaccharide material from the loricae of *Poteriochromonas* (a). The reflections are rather diffuse. They do not match the reflections given by a cotton cellulose I standard (b).

FIGURE 9 a and b X-ray diffraction pattern of the acid- and alkali-resistant material from the loricae of *Poteriochromonas* (a), extracted sequentially with 8 N HCl (2 h at 20°C), 1 N HCl (1 h at 100°C), and 5 N NaOH (1 h at 100°C). The pattern is similar to, but more distinct than, that of Fig. 8 a; cellulosic reflections are not found. Comparison with the X-ray diffraction pattern of chitin from the mucoracean fungus *Rhizopus* (b), also extracted with 5 N NaOH (2 h at 100°C), shows correspondence of most of the reflections. Only one reflection does not match the chitin pattern (arrow).

REFERENCES

1. ALLEN, M. B. 1969. Structure, physiology, and biochemistry of the *Chrysophyceae*. *Annu. Rev. Microbiol.* **23**:24-46.
2. ALLEN, D. M., and D. H. NORTHCOTE. 1975. The scales of *Chrysochromulina chiton*. *Protoplasma.* **83**:389-412.
3. ARONSON, J. M. 1965. The cell wall. In *The Fungi*. Vol. I. G. C. Ainsworth and A. S. Sussman, editors. Academic Press, Inc., New York. 49-76.
4. BARTNICKI-GARCIA, S. 1968. Cell wall chemistry, morphogenesis and taxonomy of fungi. *Annu. Rev. Microbiol.* **22**:87-108.
5. BLACKWELL, J., K.-D. PARKER, and K. M. RUDALL. 1967. Chitin fibers of the diatoms *Thalassiosira fluviatilis* and *Cyclotella cryptica*. *J. Mol. Biol.* **28**:383-385.
6. BROWN, R. M., W. W. FRANKE, H. KLEINIG, H. FALK, and P. SITTE. 1969. Cellulosic wall component produced by the Golgi apparatus of *Pleurochrysis scherffelii*. *Science (Wash. D. C.)*. **166**:894-896.
7. BROWN, R. M., W. W. FRANKE, H. KLEINIG, H. FALK, and P. SITTE. 1970. Scale formation in chrysophycean algae. I. Cellulosic and noncellulosic wall components made by the Golgi apparatus. *J. Cell Biol.* **45**:246-271.
8. BROWN, R. M., W. HERTH, W. W. FRANKE, and D. ROMANOVICZ. 1973. The role of the Golgi apparatus in the biosynthesis and secretion of a cellulosic glycoprotein in *Pleurochrysis*: a model system for the synthesis and secretion of structural polysaccharides. In *Biogenesis of Plant Cell Wall Polysaccharides*. F. Loewus, editor. Academic Press, Inc., New York. 207-257.
9. CARLSTRÖM, D. 1957. The crystal structure of α -chitin (poly-N-acetyl-D-glucosamine). *J. Biophys. Biochem. Cytol.* **3**:669-683.
10. DENNIS, D. T., and R. D. PRESTON. 1961. Constitution of cellulose microfibrils. *Nature (Lond.)*. **191**:667-668.
11. DODGE, J. D. 1973. *The Fine Structure of Algal Cells*. Academic Press, Inc., New York.
12. FOTT, B. 1971. *Algenkunde*. Gustav Fischer Verlag KG., Stuttgart, Germany.
13. FRANKE, W. W., and B. ERMEN. 1969. Negative staining of plant slime cellulose: an examination of the elementary fibril concept. *Z. Naturforsch.* **24b**:918-922.
14. FRANKE, W. W. and H. FALK. 1968. Enzymatisch isolierte Cellulosefibrillen der *Valonia*-Zellwand. *Z. Naturforsch.* **23b**:272-274.
15. FRANKE, W. W., and W. HERTH. 1973. Cell and lorica fine structure in the chrysoomonad alga, *Dinobryon sertularia* Ehr. *Arch. Mikrobiol.* **91**:323-344.
16. FREY-WYSSLING, A. 1959. *Die pflanzliche Zellwand*. Springer-Verlag KG, Berlin, Germany.
17. FREY-WYSSLING, A. 1969. The ultrastructure and biogenesis of native cellulose. *Fortschr. Chem. Org. Naturst.* **27**:1-30.
18. GARDNER, K. H., and J. BLACKWELL. 1971. The substructure of the cellulose microfibrils from the cell walls of the alga *Valonia ventricosa*. *J. Ultrastruct. Res.* **36**:725-731.
19. GÜNTHER, I. 1960. Elektronenmikroskopische Untersuchungen an der keimenden Spore von *Funaria hygrometrica*. *J. Ultrastruct. Res.* **4**:304-331.
20. HANNA, R. B., and W. A. CÔTÉ. 1974. The subelementary fibril of plant cell wall cellulose. *Cytobiologie.* **10**:102-116.
21. HERTH, W. 1974. Untersuchungen zum Aufbau und Syntheseort pflanzlicher Strukturpolysaccharide. Doctoral thesis. Faculty of Biology, University of Freiburg i. Br., Germany.
22. HERTH, W., W. W. FRANKE, H. BITTIGER, A. KUPPEL, and G. KEILICH. 1974. Alkali-resistant fibrils of β -1,3- and β -1,4-glucans: structural polysaccharides in the pollen tube wall of *Lilium longiflorum*. *Cytobiologie.* **9**:344-357.
23. HERTH, W., W. W. FRANKE, J. STADLER, H. BITTIGER, G. KEILICH, and R. M. BROWN. 1972. Further characterization of the alkali-stable material from the scales of *Pleurochrysis scherffelii*: a cellulosic glycoprotein. *Planta (Berl.)*. **105**:79-92.
24. HERTH, W., A. KUPPEL, W. W. FRANKE, and R. M. BROWN. 1975. The ultrastructure of the scale cellulose from *Pleurochrysis scherffelii* under various experimental conditions. *Cytobiologie.* **10**:268-284.
25. HEYN, A. N. J. 1966. The microcrystalline structure of cellulose in cell walls of cotton, ramie, and jute fibers as revealed by negative staining of sections. *J. Cell Biol.* **29**:181-197.
26. HEYN, A. N. J. 1969. The elementary fibril and supermolecular structure of cellulose in soft wood fiber. *J. Ultrastruct. Res.* **26**:52-68.
27. HILLIARD, D. K. 1971. Observations on the lorica structure of some *Dinobryon* species. *Oesterr. Bot. Z.* **119**:25-40.
28. HOUWINK, A. L., and D. R. KREGER. 1953. Observations on the cell walls of yeasts. An electron microscope and X-ray diffraction study. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **19**:1-24.
29. KAUSS, H. 1968. Isolierung und Fraktionierung saurer Exopolysaccharide von *Ochromonas malhamensis*. *Z. Pflanzenphysiol.* **58**:281-284.
30. KLEBS, G. 1893. Flagellatenstudien. II. *Z. Wiss. Zool.* **55**:353-445.
31. KOPECKÁ, M., H. J. PHAFF, and G. H. FLEET. 1974. Demonstration of a fibrillar component in the cell wall of the yeast *Saccharomyces cerevisiae* and its chemical nature. *J. Cell Biol.* **62**:66-76.
32. KRAMER, D. 1970. Fine structure of growing cellulose microfibrils of *Ochromonas malhamensis* Pringsheim (syn. *Poteriochromonas stipitata* Scherffel). *Z. Naturforsch.* **25b**:1017-1020.
33. KRAMER, D. 1972. Beobachtungen zur Morpho-

- genese der Zellulosehüllen von *Poteriochromonas stipitata* (Scherffel). Doctoral thesis. University of Heidelberg, Faculty of Natural Sciences, Heidelberg, Germany.
34. KREGER, D. R. 1954. Observations on cell walls of yeasts and some other fungi by X-ray diffraction and solubility tests. *Biochim. Biophys. Acta.* **13**:1-9.
 35. KREGER, D. R. 1969. Cell walls. Chap. 53. In *Handbook of Molecular Cytology*. A. Lima de Faria, editor. North Holland Res. Monographs Frontiers of Biology. A. Neuberger and E. L. Tatum, editors. Noord-Hollandsche Uity. Mij. **15**:1444-1479.
 36. KREGER, D. R., and B. J. D. MEEUSE. 1952. X-ray diagrams of *Euglena* paramylon, of the acid-insoluble glucan of yeast cell walls, and of laminaran. *Biochim. Biophys. Acta.* **9**:699-700.
 37. KRISTIANSEN, J. 1969. Lorica structure in *Chrysolykos* (*Chrysophyceae*). *Sven. Bot. Tidskr.* **64**:162-168.
 38. KRISTIANSEN, J. 1972. Studies on the lorica structure in *Chrysophyceae*. *Sven. Bot. Tidskr.* **66**:184-190.
 39. KUHL, A., and H. LORENZEN. 1964. Handling and culturing of *Chlorella*. In *Methods in Cell Physiology*. D. M. Prescott, editor. Academic Press, Inc., New York. **1**:159-187.
 40. LAMPORT, D. T. A. 1970. Cell wall metabolism. *Annu. Rev. Plant Physiol.* **21**:235-270.
 41. LAMPORT, D. T. A. 1973. The glycopeptide linkages of extensin: O-D-galactosyl serine and O-L-arabinosyl hydroxyproline. In *Biogenesis of Plant Cell Wall Polysaccharides*. F. Loewus, editor. Academic Press, Inc., New York. 149-164.
 42. LEWIN, R. A. 1962. *Physiology and biochemistry of algae*. Academic Press, Inc., New York.
 43. LEE, H. Y., and J. M. ARONSON. 1975. Composition of cellulose, the unique chitin-glucan granules of the fungus, *Apodachlya* sp. *Arch. Microbiol.* **102**:203-208.
 44. MAEDA, H., and N. ISHIDA. 1967. Specificity of binding of hexapyranosyl polysaccharides with fluorescent brighteners. *J. Biochem.* **62**:276-278.
 45. MAHADEVAN, P. P., and E. L. TATUM. 1967. Localization of structural polysaccharides in the cell wall of *Neurospora crassa*. *J. Cell Biol.* **35**:295-302.
 46. McLACHLAN, J., A. G. McINNES, and M. FALK. 1965. Studies on the chitan (chitin-poly-N-acetylglucosamine) fibers of the diatom *Thalassiosira fluviatilis* Hustedt. I. Production and isolation of chitan fibers. *Can. J. Bot.* **43**:707-713.
 47. MÜHLETHALER, K. 1967. Ultrastructure and formation of plant cell walls. *Annu. Rev. Plant Physiol.* **18**:1-23.
 48. NEVILLE, A. C., D. A. D. PARRY, and J. WOODHEAD-GALLOWAY. 1976. The chitin crystallite in arthropod cuticle. *J. Cell Sci.* **21**:73-82.
 49. NEVINS, D. J., P. D. ENGLISH, and P. ALBERSHEIM. 1968. Changes in cell wall polysaccharides associated with growth. *Plant Physiol.* **43**:914-922.
 50. OHAD, I., and D. DANON. 1964. On the dimensions of cellulose microfibrils. *J. Cell Biol.* **22**:302-305.
 51. PRESTON, R. D. 1968. Plants without cellulose. *Sci. Am.* **218**(6):102-108.
 52. PRESTON, R. D. 1974. *The physical biology of plant cell walls*. Chapman & Hall, Ltd., London.
 53. PARKER, B. C., and G. F. LEEPER. 1969. Fine structure of polysaccharide microcrystals. *Planta (Berl.)*. **87**:86-94.
 54. ROBINSON, D. G., and R. D. PRESTON. 1971. Studies on the fine structure of *Glaucozystis nostochinearum* Itzigs. I. Wall structure. *J. Exp. Bot.* **22**:635-643.
 55. RÖDERER, G. 1975. Die Wirkung von Blei auf Wachstum, Hüllenmorphogenese und Ultrastruktur von *Porteriochromonas stipitata*. Diplomarbeit. Faculty for Biology, University of Heidelberg, Germany.
 56. ROGERS, H. J., and H. R. PERKINS. 1968. Cell walls and membranes. E. & F. N. Spon, Ltd., London.
 57. RUDALL, K. M., and W. KENCHINGTON. 1973. The chitin system. *Biol. Rev. (Camb.)*. **49**:597-636.
 58. RUIZ-HERRERA, J., and S. BARTNICKI-GARCIA. 1974. Synthesis of cell wall microfibrils in vitro by a "soluble" chitin synthetase from *Mucor rouxii*. *Science (Wash. D. C.)*. **186**:357-359.
 59. RUIZ-HERRERA, J., V. O. SING, W. J. VANDERWOUDE and S. BARTNICKI-GARCIA. 1975. Microfibril assembly by granules of chitin synthetase. *Proc. Natl. Acad. Sci. (U. S. A.)* **72**:2706-2710.
 60. SCHNEPF, E. 1965. Struktur der Zellwände und Cellulosefibrillen bei *Glaucozystis*. *Planta (Berl.)*. **67**:213-224.
 61. SCHNEPF, E., G. DEICHGRÄBER, and W. KOCH. 1968. Über das Vorkommen und den Bau gestellter "Hüllen" bei *Ochromonas malhamensis* Pringsheim *O. sociabilis* nom. prov. Pringsheim. *Arch. Mikrobiol.* **63**:15-25.
 62. SCHNEPF, E., G. RÖDERER, and W. HERTH. 1975. The formation of the fibrils in the lorica of *Poteriochromonas stipitata*: tip growth, kinetics, site, orientation. *Planta (Berl.)*. **125**:45-62.